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TOWNSEND and TOWNSEND and CREW LLP

By: _____
Dana Kane

PATENT
Attorney Docket No. 020048-004200US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Byung Sook Moon
Martin Jones
Johnny Valdez

Application No.: 10/672,266

Filed: September 25, 2003

For: LYOPHILIZED BEADS CONTAINING MANNITOL

Confirmation No. 8805

Examiner: Pande, Suchira

Technology Center/Art Unit: 1637

APPELLANTS' BRIEF UNDER 37 CFR §41.37

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Further to the Notice of Appeal mailed on October 7, 2008, for the above-referenced application, Appellants appeal the final rejection of April 8, 2008. The claims on appeal have been finally rejected pursuant to MPEP §706.07(b). Accordingly, this appeal is believed to be proper.

TABLE OF CONTENTS

1. REAL PARTY IN INTEREST

The real party in interest for the above-identified application is CEPHEID, a California corporation having its principal place of business at 904 Caribbean Drive, Sunnyvale, California 94089. The assignment was recorded in the U.S. Patent and Trademark Office on January 29, 2004 at Reel 014292 / Frame 0072.

2. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences related to the present appeal.

3. STATUS OF CLAIMS

Claims 1-10, 12, 45-48, 50-53, 63 and 64 are pending. Claims 11, 13-44, 49 and 54-62 are withdrawn from further consideration as being directed to a non-elected invention. Claims 1, 2, 4-6, 13-17, 24, 25, 35-37, 44-46, 54, 55 and 60-64 have been amended.

Claims 1-10, 12, 45-48 and 50-53 were rejected under 35 U.S.C. § 112, 1st.

Claims 1-7, 10, 12, 45-48, 50, 52, 53, 63 and 64 were rejected under 35 U.S.C. § 103(a).

Claims 8 and 50 were rejected under 35 U.S.C. § 103(a).

Claims 9 and 51 were rejected under 35 U.S.C. § 103(a).

Independent claims 1, 45, 63 and 64, and dependent claims 2-10, 12, 46-48 and 50-53 are grouped together.

4. STATUS OF AMENDMENTS

Claims 1, 2, 4-6, 13-17, 24, 25, 35-37, 44-46, 54, 55 and 60-64 have been amended to place the claims in better condition for appeal.

In accordance with 37 C.F.R. §41.37(c)(1)(viii) a copy of the claims involved in the appeal are contained in the Appendix attached hereto.

5. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 1 is drawn to a lyophilized bead for use in nucleic acid amplification, where the bead includes a thermally stable enzyme and mannitol in 53% to 75% (w/w), where the bead is substantially spherical in shape (see the specification at page 2, lines 17-20; and Figure 1). Dependent claims 2-10 and 12 provide for additional bead components such as a nucleoside triphosphate or

derivative thereof, an antibody for inactivating a polymerase, a wax or oil for sequestering magnesium, HEPES, a probe, and an internal control, other ranges of mannitol including between 62% and 75% (w/w) as well as between 68% and 75% (w/w), volume of the reaction mixture for amplification, and cross-sectional size of the bead.

Independent claim 45 is drawn to a substantially spherical lyophilized bead for use in nucleic acid amplification where the bead is prepared by a process involving creating a solution of a thermally stable enzyme and mannitol, quick-freezing the solution, and freeze-drying the quick frozen solution (see the specification at page 4, lines 22-28; and Figure 1). Dependent claims 46-48 and 50-53 provide that the bead can also include a nucleoside triphosphate or derivative thereof, an antibody for inactivating a polymerase, a wax or oil for sequestering magnesium, HEPES, a probe, and an internal control, and cross-sectional size of the bead.

Independent claim 63 is drawn to a lyophilized bead for use in nucleic acid amplification, where the bead includes a thermally stable enzyme and mannitol in 53% to 75% (w/w) (see the specification at page 2, lines 17-20).

Independent claim 64 is drawn to a spherical lyophilized bead for use in nucleic acid amplification where the bead is prepared by a process involving creating a solution of a thermally stable enzyme and mannitol, quick-freezing the solution, and freeze-drying the quick frozen solution (see the specification at page 4, lines 22-28).

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Independent claims 1 and 45 along with dependent claims 2-10, 12, 46-48 and 50-53 were rejected under 35 U.S.C. § 112, 1st, as allegedly being failing to comply with the written description requirement in view of the amendment to claims 1 and 45 adding the limitation "substantially spherical."

B. Independent claims 1, 45, 63 and 64, along with dependent claims 2-7, 10, 12, 46-48 and 52-53 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Park *et al.* (U.S. Patent No. 5,861,251) in view of Treml *et al.* (U.S. Patent No. 5,763,157).

C. Dependent claims 8 and 50 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Park *et al.* (U.S. Patent No. 5,861,251) in view of Treml *et al.* (U.S. Patent No. 5,763,157) as applied to claims 1 and 45, and further in view of Kellogg *et al.* Biotechniques 1994, 16(6), 1134-1137.

D. Dependent claims 9 and 51 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Park *et al.* (U.S. Patent No. 5,861,251) in view of Treml *et al.* (U.S. Patent No. 5,763,157) as applied to claims 1 and 45, and further in view of Shively *et al.* Biotechniques 2003, 34(3), 498-504.

7. ARGUMENT

The rejections are addressed below in turn.

A. Independent claims 1 and 45 along with dependent claims 2-10, 12, 46-48 and 50-53 were rejected under 35 U.S.C. § 112, 1st, as allegedly failing to comply with the written description requirement in view of the amendment to claims 1 and 45 adding the limitation "substantially spherical."

Applicants submit that there is sufficient written description support for the limitation "substantially spherical" and that it is clear to the reader of the instant claims that Applicants had possession of the invention at the time of filing the application. Written description support for "substantially spherical" can be found in Figure 1 of the application (also shown below in paragraphs 1 and 2 of the Evidence Appendix), showing lyophilized beads having mannitol concentrations in the claimed range of between 53% and 75% (w/w) that are *substantially spherical*. The specification also provides written description support for the phrase "substantially spherical." For example, the definition of "bead" at page 7, lines 10-17 provides that "[a] bead can have a spherical as well as a nearly spherical, *e.g.*, elliptical, shape." Moreover, the definition of "lyophilized beads" at page 10, lines 23-28 provides that "[e]xemplary shapes include spherical, near spherical, elliptical or round structures." Additional support can be found in Table 4, where the beads having mannitol within the claimed range are described as having a "spherical shape" or being "smooth spherical beads."

In view of the support in the specification for beads that are substantially spherical, Applicants submit that the presently pending claims comply with the written description requirement under 35 U.S.C. § 112, 1st paragraph. For at least the foregoing reasons, withdrawal of the rejection is respectfully requested.

In the event the Examiner might consider a rejection under 35 U.S.C. § 112, 2d paragraph for alleged indefiniteness of the term "substantially spherical," Applicants submit that this term is clear to one of skill in the art. In fact, a search of issued U.S. patents on the USPTO full-text database returned more than 4,000 issued patents with the term "substantially spherical" in the claims. Accordingly, Applicants submit that the term "substantially spherical" is clear to one of skill in the art.

B. Independent claims 1, 45, 63 and 64, along with dependent claims 2-7, 10, 12, 46-48 and 52-53 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Park et al. (U.S. Patent No. 5,861,251) in view of Treml et al. (U.S. Patent No. 5,763,157).

Applicants note that the claims of the present application were previously amended to recite that the lyophilized beads of the present invention are "substantially spherical." Neither Park nor Treml describes a bead that is substantially spherical. Applicants respectfully submit that the claims of the instant invention are not obvious under 35 U.S.C. § 103(a) over Park and Treml.

Park discloses a lyophilized bead for PCR where the bead can include a DNA polymerase and a stabilizer. Park discloses many polyols useful as the stabilizer, including mannitol, where the stabilizer is present in amount of 4% by weight (see col. 4, line 24). Park, however, does not disclose a lyophilized bead having mannitol in an amount between 53% and 75% (w/w). Accordingly, the lyophilized bead of Park having 4% mannitol as a stabilizer fails to teach or disclose a bead having mannitol between 53% and 75% (w/w) of the bead, as in the instantly amended claims.

Nor are the deficiencies of Park remedied by Treml. For example, Treml discloses a variety of mono-, di-, tri- and polysaccharides, such as melezitose, cellobiose, dextranT10, maltotriose, maltose, cyclodextran, sorbitol, trehalose and sucrose. (See column 5, lines 29-31.) Nowhere, however, does Treml disclose mannitol, much less the claimed amount of mannitol. Instead, Treml teaches away from the present invention by disclosing compositions having at most 40% (w/w) of a carbohydrate[1], a carbohydrate composition that is substantially *below* the lowest claimed mannitol composition of 53% (w/w). In addition to failing to describe the claimed amount of carbohydrate, Treml also fails to describe the actual claimed carbohydrate, mannitol. Because Treml fails to describe the claimed amount of mannitol, as well as mannitol itself, Treml does not remedy the failings of Park.

Moreover, the Jones declaration submitted with the Amendment of July 12, 2007, describes unexpected and surprising results for the lyophilized mannitol beads of the present invention.

1. Substantially spherical beads are unexpectedly prepared using mannitol of between 53% and 75% (w/w)

Dr. Jones declares in paragraph 5 of the Jones declaration that the instantly claimed range of mannitol of between 53% and 75% (w/w) is a critical range for the lyophilized beads of the present invention. Within this range, the beads are reproducibly spherical (see pictures in paragraph 5 of the

[1] See column 5, lines 49-62; column 7, lines 41-64; and the Examples where 5% (w/v) of carbohydrate is equivalent to about 28% (w/w) and 10% (w/v) is equivalent to about 40% (w/w). For example, in Example 1, 10% (w/v) melezitose is about 1000 μg carbohydrate in about 2500 μg total bead weight, so 1000/2500 = 40% (w/w).

Jones declaration, shown below in paragraph 1 of the Evidence Appendix). The substantially spherical nature of the beads is inherent to the use of mannitol in the claimed range. Outside of this range, the beads can be non-spherical, and are characterized by a rough surface having pits and protrusions.

2. Mannitol beads unexpectedly afford substantially spherical beads as compared to beads of other saccharides

In paragraph 6 of the Jones declaration, Dr. Jones declares that the use of mannitol in the claimed range, rather than other saccharides or oligosaccharides, provides lyophilized beads that are reproducibly spherical. The pictures in paragraph 6 of the Jones declaration (shown below in paragraph 2 of the Evidence Appendix) show the effect on bead morphology of using trehalose versus using mannitol. The beads made using mannitol are substantially spherical (E and F). In contrast, the beads made using trehalose (A and B) form an irregular shaped mass that adheres to the bottom of the container, even where the % (w/v) of trehalose matches that of mannitol (A versus E). The beads made from trehalose did not lyophilize, and any resemblance to spherical shape by the trehalose beads prior to lyophilization was subsequently lost upon lyophilization.

3. The present invention unexpectedly provides lyophilized mannitol beads that are substantially crystalline

Dr. Jones declares in paragraph 7 (shown below in paragraph 3 of the Evidence Appendix) of the Jones declaration that lyophilized mannitol beads of the present invention, surprisingly, are substantially *crystalline* rather than glassy and amorphous. Dr. Jones further declares that lyophilized beads using compositions of the prior art are glassy and amorphous, and are thus unable to make the beads of the present invention that are reproducibly spherical. The powder x-ray diffractogram for the glassy, amorphous structure demonstrated an amorphous halo with no evidence of crystallinity. The powder x-ray diffractograms for the lyophilized beads of the invention were consistent with the δ-polymorph of crystalline mannitol. Accordingly, the lyophilized mannitol beads of the present invention demonstrate a high degree of crystallinity.

4. The use of mannitol in the claimed range unexpectedly provides lyophilized beads that are reproducibly the same size

In paragraph 8 of the Jones declaration (shown below in paragraph 4 of the Evidence Appendix), Dr. Jones declares that the surprising nature of the lyophilized beads of the present invention is also exemplified by the reproducibility and homogeneity of the size of the lyophilized beads. Using three beads from each excipient formulation of Table 1 in the instant application, bead cross-section was measured. The bead diameter data demonstrate that the lyophilized mannitol beads of the present

invention have a high degree of uniformity, as determined by the standard deviation (SD) and the coefficient of variation (%CV). The prior art trehalose beads have a %CV of around 6.5%, while the lyophilized mannitol beads of the present invention have a %CV of from 0.70 to 2.44, significantly lower than that for the trehalose beads. The higher CV numbers for the trehalose beads indicate a larger degree of variability and less reproducibility in the diameter of the trehalose beads, as compared to the mannitol beads of the present invention. Accordingly, the lyophilized mannitol beads of the present invention are surprisingly uniform, as compared to beads with a similar % w/v trehalose.

For at least the foregoing reasons, withdrawal of the rejection is respectfully requested.

C. Dependent claims 8 and 50 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Park *et al.* (U.S. Patent No. 5,861,251) in view of Treml *et al.* (U.S. Patent No. 5,763,157) as applied to claims 1 and 45, and further in view of Kellogg *et al.* Biotechniques 1994, 16(6), 1134-1137.

Claims 8 and 50 reciting hot start methods are rejected as obvious over Park, Treml and Kellogg. Park and Treml are relied upon as set forth above, and Kellogg is cited for disclosure of the polymerase antibody technique of hot starting PCR. In response, applicants rely on the arguments set forth above for claims 1-8, 10, 12, 45-48, 50 and 52-53. Claims 8 and 50 are dependent upon claim 1, and claim 1 is non-obvious for the reasons set forth above. Treml and Park fail to render PCR beads with mannitol as the major excipient obvious, and adding Kellogg disclosing hot start antibodies to the reference combination does not cure that failure.

D. Dependent claims 9 and 51 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Park *et al.* (U.S. Patent No. 5,861,251) in view of Treml *et al.* (U.S. Patent No. 5,763,157) as applied to claims 1 and 45, and further in view of Shively *et al.* Biotechniques 2003, 34(3), 498-504.

Claims 9 and 51 reciting buffering components of the PCR mix are rejected as obvious over Park, Treml and Shively. Park and Treml are relied upon as set forth above, and Shively is cited for disclosure of HEPES for use in PCR. In response, applicants rely on the arguments set forth above for claims 1-8, 10, 12, 45-48, 50 and 52-53. Claims 9 and 51 are dependent upon claim 1, and claim 1 is non-obvious for the reasons set forth above. Treml and Park fail to render PCR beads with mannitol as the major excipient obvious, and adding Shively disclosing HEPES to the reference combination does not cure that failure.

8. CONCLUSION

For these reasons, it is respectfully submitted that the rejections should be reversed.

Respectfully submitted,

Alexander R. Trimble
Reg. No. 52,301

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415 576-0200
Fax: 415 576-0300
61906789 v1

9. CLAIMS APPENDIX

1. (Currently Amended): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, said lyophilized bead being substantially spherical in shape and comprising:

a thermally stable enzyme; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between ~~about~~ 53% and ~~about~~ 75% (w/w).

2. (Currently Amended): The lyophilized bead of claim **1**, wherein said amplification occurs in a reaction mixture comprising a volume of between ~~about~~ 5 μL and ~~about~~ 200 μL.

3. (original): The lyophilized bead of claim **1**, further comprising a nucleoside triphosphate or a derivative thereof.

4. (Currently Amended): The lyophilized bead of claim **1**, wherein said lyophilized bead has an average cross-section of between ~~about~~ 1 millimeter and ~~about~~ 4.5 millimeters.

5. (Currently Amended): The lyophilized bead of claim **1**, wherein said weight percentage is between ~~about~~ 62% and ~~about~~ 75% (w/w).

6. (Currently Amended): The lyophilized bead of claim **5**, wherein said weight percentage is between ~~about~~ 68% and ~~about~~ 75% (w/w).

7. (original): The lyophilized bead of claim **1**, wherein said thermally stable enzyme is selected from the group consisting of polymerase, ligase, and combinations thereof.

8. (previously presented): The lyophilized bead of claim **1**, further comprising a component selected from the group consisting of an antibody that inactivates a polymerase and a wax or oil to sequester magnesium.

9. (Previously Presented): The lyophilized bead of claim **1**, further comprising N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES).

10. (original): The lyophilized bead of claim **1**, further comprising a probe.

11. (withdrawn): The lyophilized bead of claim **1**, further comprising a reverse transcriptase.

12. (original): The lyophilized bead of claim **1**, further comprising an internal control.

13. (withdrawn - Currently Amended): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, said lyophilized bead comprising:

a forward polynucleotide primer;

a reverse polynucleotide primer; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between ~~**about**~~ 53% and ~~**about**~~ 75% (w/w).

14. (withdrawn - Currently Amended): The lyophilized bead of claim **13**, wherein said amplification occurs in a reaction mixture comprising a volume of between ~~**about**~~ 5 μL and ~~**about**~~ 200 μL.

15. (withdrawn - Currently Amended): The lyophilized bead of claim **13**, wherein said lyophilized bead has an average cross-section of between ~~**about**~~ 1 millimeter and ~~**about**~~ 4.5 millimeters.

16. (withdrawn - Currently Amended): The lyophilized bead of claim **13**, wherein said weight percentage is between ~~**about**~~ 62% and ~~**about**~~ 75% (w/w).

17. (withdrawn - Currently Amended): The lyophilized bead of claim **16**, wherein said weight percentage is between ~~**about**~~ 68% and ~~**about**~~ 75% (w/w).

18. (withdrawn): The lyophilized bead of claim **13**, further comprising HEPES.

19. (withdrawn): The lyophilized bead of claim **13**, further comprising a probe.

20. (withdrawn): The lyophilized bead of claim **13**, further comprising an internal control.

21. (withdrawn): The lyophilized bead of claim **13**, wherein said nucleic acid sequence is selected from the group consisting of bacterial, fungal, and viral nucleic acid sequences.

22. (withdrawn): The lyophilized bead of claim **21**, wherein said bacterial nucleic acid sequence is derived from a member selected from the group consisting of *Bacillus Anthracis, Yersinia pestis, Clostridium botulinum, Francisella tularensis*, Group B *Streptococcus*, *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Xylella fastidiosa.*

23. (withdrawn): The lyophilized bead of claim **21**, wherein said viral nucleic acid sequence is derived from a member selected from the group consisting of Vaccinia, West Nile Fever virus, Equine Encephalitis virus, and Foot and Mouth Disease virus.

24. (withdrawn - Currently Amended): A method for the amplification of a nucleic acid sequence, said method comprising:

(a) dissolving a lyophilized bead in a liquid, wherein said lyophilized bead comprises:

a thermally stable enzyme; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between ~~about~~ 53% and ~~about~~ 75% (w/w), thus forming a reaction mixture; and

(b) subjecting said reaction mixture to an amplification reaction.

25. (withdrawn - Currently Amended): The method of claim **24**, wherein said reaction mixture further comprises a volume of between ~~about~~ 5 μL and ~~about~~ 200 μL.

26. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a nucleoside triphosphate or a derivative thereof.

27. (withdrawn): The method of claim **24**, wherein said thermally stable enzyme is selected from the group consisting of polymerase, ligase, and combinations thereof.

28. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a forward polynucleotide primer.

29. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a reverse polynucleotide primer.

30. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a probe.

31. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a nucleic acid comprising said nucleic acid sequence.

32. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises HEPES.

33. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises an internal control.

34. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a hot start methodology.

35. (withdrawn): The method of claim **24**, wherein said lyophilized bead has an average cross-section of between ~~**about**~~ 1 millimeter and ~~**about**~~ 4.5 millimeters.

36. (withdrawn - Currently Amended): A method for the amplification of a nucleic acid sequence, said method comprising:

(a) dissolving a lyophilized bead in a liquid, wherein said lyophilized bead comprises:

a forward polynucleotide primer;

a reverse polynucleotide primer; and

mannitol; and

wherein said lyophilized bead has a weight percentage of said mannitol of between ~~**about**~~ 53% and ~~**about**~~ 75% (w/w), thus forming a reaction mixture; and

(b) subjecting said reaction mixture to an amplification reaction.

37. (withdrawn - Currently Amended): The method of claim **36**, wherein said reaction mixture further comprises a volume of between ~~**about**~~ 5 μL and ~~**about**~~ 200 μL.

38. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a nucleoside triphosphate or a derivative thereof.

39. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a probe.

40. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a nucleic acid comprising said nucleic acid sequence.

41. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises HEPES.

42. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a thermally stable enzyme.

43. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises an internal control.

44. (withdrawn - Currently Amended): The method of claim **36**, wherein said lyophilized bead has an average cross-section of between ~~**about**~~ 1 millimeter and ~~**about**~~ 4.5 millimeters.

45. (Currently Amended): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, said lyophilized bead being substantially spherical and prepared by a process comprising:

(a) creating an aqueous solution, said aqueous solution comprising:

a thermally stable enzyme; and

mannitol;

wherein said solution has a concentration of said mannitol between ~~**about**~~ 0.38 M (moles of mannitol/liter of solution) and ~~**about**~~ 0.99 M (moles of mannitol/liter of solution);

(b) quick-freezing the product of (a); and

(c) freeze-drying the product of (b).

46. (Currently Amended): The lyophilized bead of claim **45**, wherein the product of (c) has an average cross-section of between ~~**about**~~ 1 millimeter and ~~**about**~~ 4.5 millimeters.

47. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a nucleoside triphosphate or a derivative thereof.

48. (original): The lyophilized bead of claim **45**, wherein said thermally stable enzyme is selected from the group consisting of polymerase, ligase, and combinations thereof.

49. (withdrawn): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a reverse transcriptase.

50. (previously presented): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a component selected from the group consisting of an antibody that inactivates a polymerase and a wax or oil to sequester magnesium.

51. (Previously Presented): The lyophilized bead of claim **45**, wherein the product of (c) further comprises N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES).

52. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a probe.

53. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises an internal control.

54. (withdrawn - Currently Amended): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, prepared by a process comprising:

(a) creating an aqueous solution, said aqueous solution comprising:

a forward polynucleotide primer;

a reverse polynucleotide primer; and

mannitol;

wherein said solution has a concentration of said mannitol between ~~about~~ 0.38 M (moles of mannitol/liter of solution) and ~~about~~ 0.99 M (moles of mannitol/liter of solution);

(b) quick-freezing the product of (a); and

(c) freeze-drying the product of (b).

55. (withdrawn - Currently Amended): The lyophilized bead of claim **54**, wherein the product of (c) has an average cross-section of between ~~about~~ 1 millimeter and ~~about~~ 4.5 millimeters.

56. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises a nucleoside triphosphate or a derivative thereof.

57. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises HEPES.

58. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises a probe.

59. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises an internal control.

60. (withdrawn - Currently Amended): A lyophilized bead suitable for use in microanalytic systems comprising:

a moisture-sensitive reactant; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between ~~about~~ 53% and ~~about~~ 75% (w/w); and

wherein said lyophilized bead has an average cross-section of between ~~about~~ 1 millimeter and ~~about~~ 4.5 millimeters.

61. (withdrawn - Currently Amended): The lyophilized bead of claim **60**, wherein said weight percentage is between ~~about~~ 62% and ~~about~~ 75% (w/w).

62. (withdrawn - Currently Amended): The lyophilized bead of claim **60**, wherein said weight percentage is between ~~about~~ 68% and ~~about~~ 75% (w/w).

63. (Currently Amended): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, said lyophilized bead comprising:

a thermally stable enzyme; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between ~~about~~ 53% and ~~about~~ 75% (w/w).

64. (Currently Amended): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, prepared by a process comprising:

(a) creating an aqueous solution, said aqueous solution comprising:

a thermally stable enzyme; and

mannitol;

wherein said solution has a concentration of said mannitol between ~~about~~ 0.38 M (moles of mannitol/liter of solution) and ~~about~~ 0.99 M (moles of mannitol/liter of solution);

(b) quick-freezing the product of (a); and

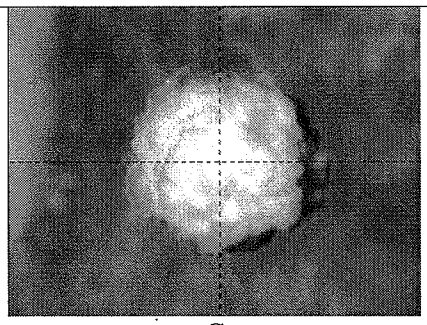
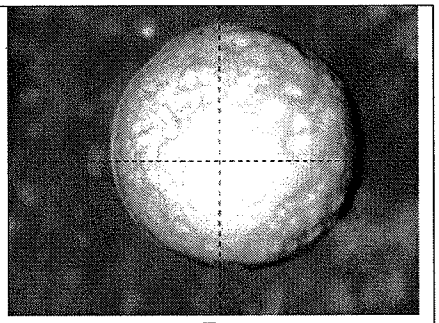
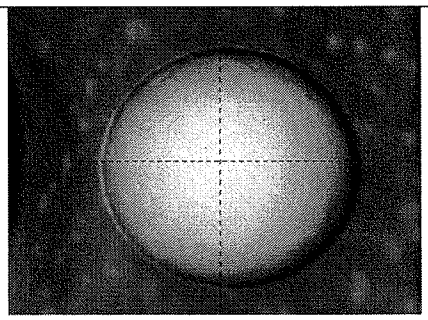
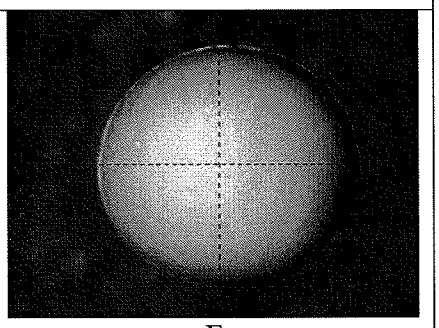
(c) freeze-drying the product of (b).

10. EVIDENCE APPENDIX

The following evidence was originally submitted July 12, 2007 in a declaration under 37 CFR 1.132 by Martin Jones, Ph.D. ("the Jones declaration") in order to provide evidence of surprising and unexpected results for the lyophilized beads of the present invention.

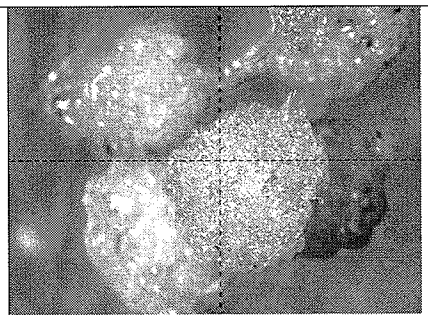
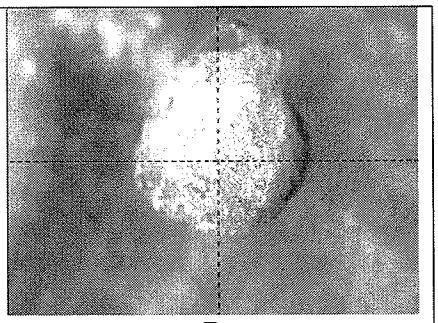
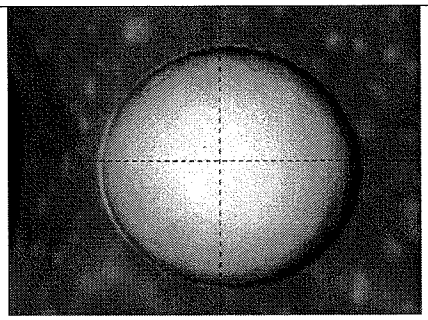
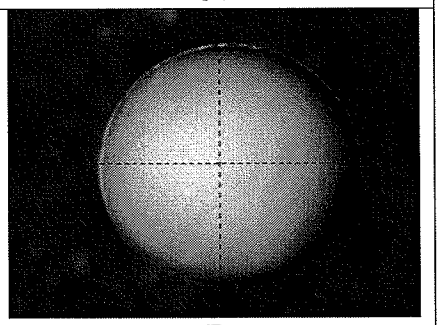
1. Paragraph 5 of the Jones Declaration

5. The claimed range of between about 53% and about 75% (w/w) of mannitol is a critical range for the lyophilized beads of the present invention. Inside this range, the beads are reproducibly spherical with a smooth morphology. Outside of this range, the beads can be non-spherical, and characterized by a rough surface having pits and protrusions. The pictures below (Figure 1 of the application) demonstrate the surprising advantage of using mannitol in the claimed range of between about 53% and about 75% (w/w). At 43% (w/w) mannitol (C), the bead is semi-spherical, but characterized by sharp protrusions forming a rough surface. At 50% (w/w) mannitol (D), the bead is spherical, but with many pits and craters on the surface (visible as the white spots). At 60% (w/w) mannitol (E), within the claimed range, the beads are spherical and smooth. Beads at 65% (w/w) mannitol (F) are also spherical and smooth. As one of skill in the art, it is surprising that beads having mannitol in the claimed range of between about 53% and about 75% (w/w) are reproducibly spherical with a smooth morphology, as compared to beads having mannitol outside the claimed range.

	 C	 D
Saccharide	Mannitol	Mannitol
% (w/v)	4.5	6.0
% (w/w)	**43**	**50**
	 E	 F
Saccharide	Mannitol	Mannitol
% (w/v)	9.0	11.0
% (w/w)	**60**	**65**

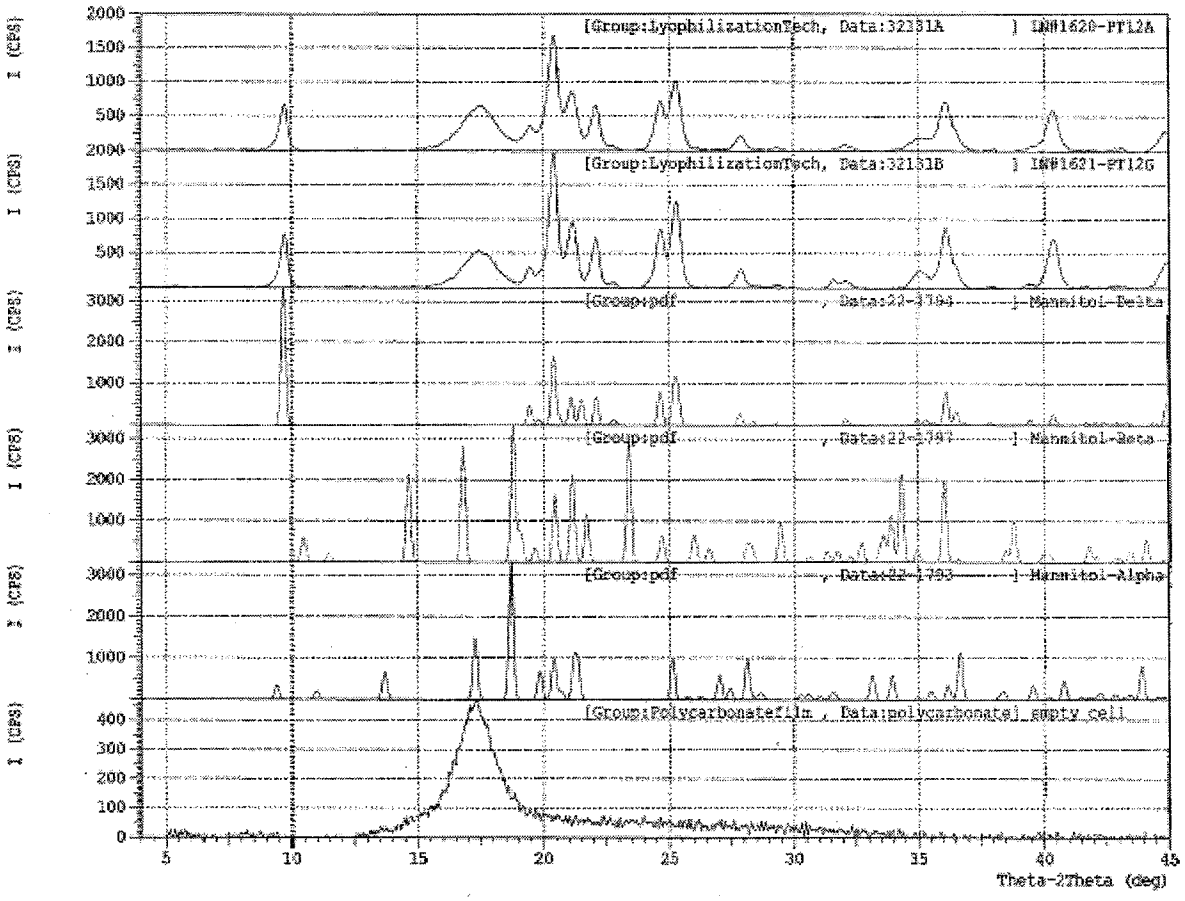
2. Paragraph 6 of the Jones Declaration

6. The use of mannitol rather than other saccharides or oligosaccharides is also a critical feature of the present invention. The use of mannitol in the claimed range provides beads that are reproducibly spherical with a smooth morphology. The pictures below (Figure 1 of the application) show the effect on bead morphology of using trehalose versus using mannitol. The beads made using mannitol are smooth and spherical (E and F). In contrast, the beads made using trehalose (A and B) form a shiny, clear, irregular shaped mass that adheres to the bottom of the container, even where the % (w/v) of trehalose matches that of mannitol (A versus E). The beads made from trehalose did not lyophilize, and any resemblance to spherical shape by the trehalose beads prior to lyophilization was subsequently lost upon lyophilization. As one of skill in the art, it is also surprising that exchanging mannitol for trehalose at similar % w/v, affords reproducibly spherical beads having a smooth morphology.

	 A	 B
Saccharide	**Trehalose**	**Trehalose**
% (w/v)	9.0	18.8
% (w/w)	72	84
	 E	 F
Saccharide	**Mannitol**	**Mannitol**
% (w/v)	9.0	11.0
% (w/w)	60	65

3. Paragraph 7 of the Jones Declaration

7. The lyophilized mannitol beads of the present invention, surprisingly, are substantially *crystalline* rather than glassy and amorphous. Lyophilized beads of the prior art are glassy and amorphous, and are thus unable to make the beads of the present invention that are reproducibly spherical with a smooth morphology. Bead crystallinity was assessed using powder x-ray diffraction (PXRD) (see Example 2 of the application). In the figure below, the first two diffractograms are of lyophilized mannitol beads of the present invention. Diffractograms 3, 4 and 5 are diffractograms of the δ, β and α crystal polymorphs of mannitol. The last diffractogram is of an empty cell. The powder x-ray diffractograms for the lyophilized beads of the present invention are consistent with the δ-polymorph of crystalline mannitol. X-ray diffractograms of glassy, amorphous beads demonstrate an amorphous halo with no evidence of crystallinity. Accordingly, the lyophilized mannitol beads of the present invention surprisingly demonstrate a high degree of crystallinity, as compared to other lyophilized beads.



4. Paragraph 8 of the Jones Declaration

8. The surprising nature of the lyophilized beads of the present invention is also exemplified by the reproducibility and homogeneity of the size of the lyophilized beads. Using three beads from each excipient formulation of Table 1 in the application, bead cross-section was measured using the See-Brez Video Microscope (Quality Control Solutions Inc., Temecula, CA). Each bead was measured twice, for a total of six measurements per excipient formulation. The bead diameter data below (Table 3 in the application) demonstrate that the lyophilized mannitol beads of the present invention have a high degree of uniformity, as determined by the standard deviation (SD) and the coefficient of variation (%CV). The %CV is the ratio of the standard deviation to the mean, and allows comparison of the variation of populations that have significantly different mean values. The trehalose beads have a %CV of around 6.5%, while the lyophilized mannitol beads of the present invention have a %CV of from 0.70 to 2.44, significantly lower than that for the trehalose beads. The higher %CV numbers for the trehalose beads indicate a larger degree of variability and less reproducibility in the diameter of the trehalose beads, as compared to the mannitol beads. Accordingly, the lyophilized mannitol beads of the present invention are surprisingly uniform, as compared to beads with a similar % w/v trehalose.

	Trehalose % w/v		**Mannitol % w/v**					
	18.8	**9.0**	**4.5**	**6.0**	**7.0**	**8.0**	**9.0**	**11.0**
Unit	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
Bead #1	2.2890	1.8260	2.1780	2.8190	2.8195	2.9320	2.8671	2.8700
	2.5430	1.7715	2.2020	2.8245	2.7595	2.8635	2.7145	2.8435
Bead #2	2.4470	2.0160	2.1515	2.7605	2.6375	2.8010	2.8365	2.8865
	2.1675	2.0710	2.2170	2.7365	2.7095	2.7840	2.7149	2.8300
Bead #3	2.1875	1.8212	2.2130	2.8835	2.6655	2.8590	2.8265	2.8570
	2.2450	1.7900	2.2315	2.7465	2.7500	2.8585	2.7700	2.8500
Mean	2.31	1.88	2.20	2.80	2.72	2.85	2.79	2.86
SD	0.15	0.13	0.03	0.06	0.07	0.05	0.07	0.02
%CV	**6.50**	**6.77**	**1.33**	**2.04**	**2.44**	**1.84**	**2.33**	**0.70**
N	6	6	6	6	6	6	6	6

11. RELATED PROCEEDINGS APPENDIX

none